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The structural properties of 5-methyl-2-phenyl-2H-1,2,3-triazole-4carboxylic acid and chromogenic mechanism on its rhodamine B derivatives to Hg²⁺ ions

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ABSTRACT

5-Methyl-2-phenyl-2H-1,2,3-triazole-4-carboxylic acid (MPTC), a newly synthesized compound, was explored to study the structural properties and theoretical spectra by using GaussView5.0 program package and the time dependent density functional theory (TD DFT). The calculated quantum chemical values suggested that it is easy for MPTC to lose electron with weak electron accepting ability. And the results of experimental measurements on fluorescence and absorption spectra were consistent with that of the calculated spectra in great degree. In addition, MPTC was successfully used and synthesized a novel rhodamine B derivative RMPTC containing 1,2,3-triazole unit. It is found that there is special chromogenic response of RMPTC to Hg²⁺ ions in N, N-dimethylformamide (DMF)-H₂O (v/v=1/1, Tris-HCl, pH 7.4) with the triazole appended colorless chemosensor turned to pink and enabled naked-eye detection. The fluorescence signal for RMPTC-Hg²⁺ system was not affected by other coexisting metal ions. The 1:2 stoichiometric structure of RMPTC and Hg²⁺ is confirmed using a Job's plot estimation and TD DFT calculations. The corresponding "off-on" fluorescence mechanism of RMPTC binding to Hg²⁺ which were ascribed to Hg²⁺ inducing the ring-opened rhodamine B moiety were proposed. This study was an advancement for the application of 1,2,3-triazole compound in photophysical chemistry field and provides guidance for exploring simple and high-selectivity Hg²⁺ probes in aqueous solutions under physiological conditions.

Key words: 5-Methyl-2-phenyl-2H-1,2,3-triazole-4-carboxylic acid, structural properties, rhodamine B, synthesis, chromogenic mechanism, Hg^{2+} ion.

1. Introduction

Since the landmark research on the synthesis of 1,2,3-triazoles in 2002[1], these

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compounds have been receiving permanent interest due to their unique chemical structure and physicochemical properties. 1,2,3-Triazoles are five member N-heterocyclic compounds and are easy to conjugate or combine with other substituent groups to form multiple types of derivatives. Furthermore, nitrogen heterocycles with triazole ring have been widely applied in different fields of chemical, biological, macromolecular materials and pharmaceutical sciences because of their less toxic effects and good biological activity[2-3]. Some studies have also shown that 1,2,3-triazoles or their derivatives can act as ligands capable of interaction with various metal ions and have exhibited specific pharmacological action[4-6]. However, it is well known that the lack or overdose of metal ions will lead to the physiologic disturbance of organisms. So it is especially practical to design and synthetize a novel compound containing 1,2,3-triazole as a metal ion probe. For instance, as one of the toxic metal ions, mercury ion (Hg²⁺) is one of the most poisonous species to human beings due to the strong interaction of Hg^{2+} with sulfhydryl groups of proteins in a wide variety of organs including brain, kidneys, central nervous system and immune system. After sinking in water, mercury ions can accelerate accumulation by the aquatic food chain. The contamination of mercury ions has been becoming one of the most serious problems in the environment because of the increasing industrial and agricultural activities as well as the improper release of metal ions from waste waters and domestic effluents[7-8]. How to detect mercury ions efficiently and sensitively has aroused the more and more concern of researchers. And enormous studies have been carried out to develop a wide variety of analytical techniques for Hg²⁺ detection [9-11]. Based on the broad chemical versatility and biological activity of 1,2,3-triazoles, there is still an unmet demand to develop and make full use of new 1,2,3-triazoles for highly sensitive and selective detection of Hg^{2+} in environmental and biological samples.

The rhodamine-based fluorophores have attracted considerable interest due to their excellent spectroscopic properties such as high fluorescence quantum yield, broad absorption, large extinction coefficient, broad emission wavelength, and high light stability [12]. Generally, the rhodamine B derivatives could show significant fluorescence enhancement and color variations from the closed loop state turned to be open loop state when binding to the ligands. So it has been widely applied to design as "off-on" fluorescent probes for detecting metal ions[13]. Recent studies on the fluorescence probes obtained from rhodamine-triazoles systems have shown that these

complexes could response specifically to some metal ions such as Zn^{2+} , Hg^{2+} , Cu^{2+} , Al³⁺ etc [14-16]. Among different research techniques for detection of metal ions, the more common methods include inductively coupled plasma mass spectrometry (ICP-MS)[17], atomic absorption spectroscopy(AAS) [18], fluorescence spectroscope [19] and electrochemistry [20] etc. Especially the color change is a simple but extremely practical and economic tool to recognize and detect analytical species. Compared with fluorescence channels, it worked in the natural light conditions without excitation of extraneous light. Therefore the fluorescence analysis method with the colorimetric sensors have attracted great attention due to the advantages of easy operation, signal visibility (instrument-free), fast response, applicable for on site and high throughput analysis[21-22]. In present work, 5-methyl-2-phenyl -2H-1,2,3-triazole-4-carboxylic acid (MPTC, Scheme 1) was a new compound synthesized under copper-catalyzed oxidative conditions[23]. Molecular properties for ionization potential, electron affinity, molecular orbitals MPTC like and theoretical spectra have been calculated by using TD-DFT method with HF/6-31G(d) and B3LYP/6-31G(d) basis set. Then MPTC was designed and introduced to rhodamine B to synthesize a novel derivative named RMPTC, which exhibited excellent selectivity toward Hg²⁺ with chromogenic reaction over other metal ions and can be applied to detect Hg^{2+} ions qualitatively under physiological conditions.

2. Experimental

2.1. Materials and Instrumentations

MPTC was provided by Organic Chemistry Lab of Hainan Normal University, China, and the stock solution $(5.0 \times 10^{-4} \text{mol/L})$ was prepared in methanol. Tris-HCl buffer was selected to keep the pH of the solution at 7.4. Sodium hydroxide (NaOH) and chloride salts including Pb²⁺, Mn²⁺, K⁺, Na⁺, Ag⁺, Ca²⁺, Cd²⁺, Co²⁺, Cu²⁺, Fe³⁺, Zn²⁺, Ni²⁺, Hg²⁺, Li⁺ and Mg²⁺ were acquired from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All other reagents were of analytical reagent grade and obtained commercially without further purification. Double distilled water was used throughout the experiment.

NMR spectra were measured at room temperature on a Bruker Avance 400MHz spectrometer at 400 MHz in CDCl₃. Fluorometric experiments were carried out on a RF-5301PC spectrofluorophotometer (Shimadzu, Japan) and a F-7000 spectrofluorophotometer (Hitachi, Japan) equipped with a xenon lamp source and 1.0

cm quartz cells. A thermostatic water bath was used to keep the reaction systems at a constant temperature. The UV-vis absorbance spectra were recorded on a Hitachi U3900/3900H spectrophotometer (Hitachi, Japan) equipped with 1.0 cm quartz cells. *2.2 Synthesis*

According to the reported procedure [24], the first step is to synthesize B hydrazide **1** as shown in Scheme 1. Rhodamine B (1.0 g, 2.1 mmol) was dissolved in 20 mL ethanol in a 50mL flask, 80% hydrazine hydrate (2 mL) was added with stirring at room temperature and the stirred mixture was heated to reflux for 3 h. Then 50 mL water was added to the mixture and extracted with 50 mL ethyl acetate three times. The combined organic layer was dried over anhydrous sodium sulfate and then filtered, affording intermediate **1** as a yellow solid (0.52 g, 54.74%); ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 1.16 (t, J = 6.8 Hz, 12H),3.34 (q, J = 7.2 Hz, 8H), 3.61 (s, 2H), 6.30 (m, 2H), 6.45(m, 4H), 7.1 (m, J = 2.64 Hz, 1H), 7.44 (m, 2H), 7.94(m, 1H); ¹³CNMR (400 MHz, CDCl₃) δ (ppm) = 166.03, 153.75, 151.45, 148,78,132.39, 129.91, 128.00, 127.95, 123.71, 122.83, 107.94, 104.47, 97.89, 77.32, 77.00, 76.68, 65.82, 44.25, 12.50.

The second step is the synthesis of RMPTC as follows: A mixture of MPTC (100mg, 0.49 mmol) and a solid of 2.3mL SOCl₂ in a 10 mL round-bottomed flask was heated to reflux for 3 h. Then the reflux device was substituted by the distillation device and the residuary SOCl₂ was distilled out to give the product 2, after which, the product 2, the solution of intermediate 1 (234mg, 0.51mmol), 10mL dichloromethane and 1mL ethylenediamine were mixed and stirred at room temperature for 12h then extracted with ethyl acetate and H₂O. When the reaction was completed the organic phase was washed three times by deionized water and dried over Na₂SO₄. The solvent was evaporated and the obtained solid was dried and purified by a silica column chromatography (elute: petroleum ether: ethyl acetate=4:1). The target product RMPTC was obtained as pale yellow solid in 37.01% yield and 116.3mg. ¹H NMR (400 MHz, CDCl₃) & 7.99 (s, 1H), 7.92 (s, 1H), 7.90 (s, 1H), 7.54-7.46 (m, 2H), 7.40 (t, J = 7.7 Hz, 2H), 7.32 (d, J = 7.2 Hz, 1H), 7.16 (d, J = 6.9 Hz, 1H), 6.77 (s, 1H), 6.75 (s, 1H), 6.38 (s, 1H), 6.36 (s, 3H), 3.33 (dd, J = 13.7, 6.7Hz, 8H), 2.48 (s, 3H), 1.15 (t, J = 7.0 Hz, 12H);¹³C NMR (100 MHz, CDCl₃) δ 165.23, 158.65, 153.73, 151.64, 148.96, 148.15, 139.15, 138.06, 133.16, 129.27, 129.16, 128.95, 128.26, 127.91, 124.08, 123.51, 119.06, 108.11, 104.52, 97.80, 66.24, 44.32, 12.54, 11.08. HRMS (ESI) (*m/z*): [M+H]⁺ calcd. for C₃₈H₄₀N₇O₃, 642.3193; found,

642.3199.

2.3 Quantum chemical calculation of MPTC

GaussView5.0 program package was used to fully optimize the molecular structure of MPTC in the ground state at Hartree-Fock Restricted algorithm level. The molecular orbital was analyzed by the data obtained from HF/6-31G (d) method. The electronic properties of MPTC including frontier molecular orbital LUMO, HOMO energies, and their band gap were determined to show the effect of electron density transfer within the molecule. Then the values of ionization potential and electron affinity of MPTC were obtained by Gaussian 09 software at the DFT//B3LYP/6-31G* algorithm level. The theoretical absorption spectra of MPTC were performed by using the time dependent density functional theory (TD DFT). The Configuration Interaction with Single excitations (CIS) method was used to fully optimize the configurations of lowest excited state. Based on the above, the theoretical fluorescence emission spectra of MPTC was obtained from TD DFT [25]. Additionally, based on Gaussian 09 program package, B3LYP method was applied to fully optimize the molecular structure of RMPTC and calculate the structural energy in terms of LANL2TZ basis set for Hg and 6-311G(d) basis set for H, C, N, O and F respectively[26].

2.4 Ions detection experiments of RMPTC

The different concentration solutions of RMPTC and Hg^{2+} ions were prepared by stepwise dilution of stock solutions (0.5 mM for RMPTC and 5mM for Hg^{2+}) in acetonitrile and water, respectively. The test solution of RMPTC was diluted with DMF-water (v/v=1:1, Tris–HCl, pH=7.40) buffer solution to give a concentration of 1×10^{-5} mol/L. The fluorescence and UV-vis absorption spectra titrations were performed by adding the corresponding volume of metal ions to the test solution of RMPTC. The excitation wavelength of fluorescence measurement is fixed at 563 nm and that of emission wavelength at 584nm respectively.

To evaluate the selectivity of RMPTC for Hg^{2+} detection, a series of competitive metal ions, including Pb^{2+} , Mn^{2+} , K^+ , Na^+ , Ag^+ , Ca^{2+} , Cd^{2+} , Co^{2+} , Cu^{2+} , Fe^{3+} , Zn^{2+} , Ni^{2+} , Hg^{2+} , Li^+ and Mg^{2+} , at a final concentration of 500 nM, were tested as following the same experimental procedure. All spectroscopic titration measurements were repeated at least three times until consistent values were obtained.

3. Results and discussion

3.1 Molecular Geometry of MPTC

Quantum chemical calculation method such as the time dependent density functional theory (TD DFT) has become a powerful tool in the investigation of the molecular structure and optical properties and are finding increasing use in application related to biological systems[27]. The calculation of ground-state geometry optimization of MPTC obtained from GaussView5.0 software at the Hatree-Fock Restricted algorithm level is shown in Fig. 1A, which indicated that the structure of MPTC is non planar with various groups. The compound MPTC consists of 24 atoms and the geometrical parameters of MPTC including bond length and bond angle are listed in Table S1 by using DFT/B3LYP method with HF/6-31G(d), CIS/6-31G(d), and B3LYP/6-31G(d) as the different basis sets respectively. It is found that the values of calculated bond lengths and bond angles are similar at all levels of calculations. The bond length varied from 0.95 A to 1.49A. The bond angle varied from 104.09 to 130.56°. But the dihedrals show relatively small differences due to using various computing methods. It is noteworthy that the values of the C-C bond length in benzene ring (marked 7-12) were between 1.35 and 1.44 Å for three different basis sets, which are much shorter than the typical C-C single bond (1.54 Å), longer than the C=C double bond (1.34 Å) and shorter than that of normal C-N bond (1.47 Å)[28]. The results on the bond lengths and angles indicated that the molecule of MPTC is no planar.

3.2 Frontier Molecular Orbital Analysis

The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) known as Frontier Molecular Orbitals (FMO's) are two of the most important tools used in quantum chemistry. According to FMO's theory, the formation of a transition state is due to the interaction between HOMO and LUMO levels of the reacting species. In general, LUMO can be considered as the innermost orbital which containing free places representing the ability to accept an electron, while HOMO can be thought as the outermost orbital which contains electrons representing the ability to donate an electron. The lower the LUMO energy, the easier the acceptance of electrons from electron-donating substitutes. Energy of HOMO is directly related to the ionization potential and LUMO energy is directly related to the electron affinity[29-30]. Fig. B1 and Fig. B2 displayed the lowest unoccupied molecular orbital (LUMO, 0.08028ev) and highest occupied molecular orbital (HOMO, -0.3214ev) of MPTC respectively. The other information on molecular

orbital were provided in Table S2 and Fig. S1 Supplemental Materials. From Table 2s, it indicated that the values of LUMO is higher than that of HOMO, which means that the acceptance of electrons of MPTC from other electron donating group is not easy to obtain. The energy difference between the HOMO and LUMO orbitals is called the energy gap which serves as an important stability factor for the compound [31]. The value of HOMO-LUMO energy gap for MPTC is 0.4017ev, which means that the molecule has stable structure. And more easily electrons are excited from the ground state to excited state due to lower the energy gap, which is proved by the following obtained from the spectroscopic experiments. In addition, results some physicochemical parameters were calculated such as vertical ionization potential (6.377 eV), adiabatic ionization potential (6.572 eV), vertical electron affinitie(1.804 eV) and adiabatic electron affinity (2.073 eV) by using the diffuse functions B3LYP/6-311G+(d) at the DFT//B3LYP/6-31G* algorithm level. These values suggested that it is easy for MPTC to lose electron or the electron accepting ability of MPTC is very weak [32].

3.3 Spectra analysis of MPTC

Since the HOMO-LUMO gap is also a crucial factor related to the luminescent properties[31, 33]. In the present study, the calculated results indicated that the electron transition of MPTC mainly focused on HOMO-JLUMO transition, which suggested that MPTC had luminescent properties and confirmed by the spectroscopic measurements. Fig. 2A showed the predicted electronic absorption spectra of MPTC by TD-DFT calculations. The inset in Fig. 2A is the absorption spectra of MPTC in methanol. It can be seen that both of two graphs showed the similar shape with double peaks, but the positions of their absorption maximum bands are different. In principle, the absorption band observed at about 280 nm was more likely to attribute to the formation of polyene π bonds or π - π * transition by a triazole ring and benzene ring of MPTC. On the other hand, the increase of solvent polarity accounts for the shift of absorption band towards the shorter wavelength direction due to $n-\pi^*$ transition. It is possible that MPTC with the nitrogen atoms as lone electron pair coordinated to methanol solvent via hydrogen bonds, which increased the energy gap between ground state and excited state. So it needed greater increased energy supply for $n-\pi^*$ transition and resulted in the blue-shift effect [34]. Therefore, it is noticed that the maximum absorption peak is at 286 nm by TD DFT calculations, which is in line

with the strongest absorption peak at 277 nm obtained from the experimental measurement for MPTC.

Fig.2 (B) showed the emission spectra by TD DFT methods and that of measured in methanol (plot "a" in the inset of Fig. 2 (B)). The calculated spectra exhibited the maximum fluorescence emission peak at 365nm, which has some difference in comparison with the experimental data measured at 346nm. The reasonable explanation is that the conjugated aromatic compound MPTC caused $\pi \rightarrow \pi^*$ transition from ground state to excited state. Since the polarity of excited state is stronger than that of ground state and low polar solvent can have more influence on stabilizing the ground state. So the position of maximum fluorescence emission shifted towards the longer wavelength side [35]. However, methanol, a high polar molecule, was used as a solvent of MPTC and had more effect on stabilizing the excited state. Therefore produced the blue-shift effect with the change of the maximum fluorescence emission peak from 365 nm to 346 nm.

Hence, the theoretical results on the UV-Vis absorption and fluorescence spectra by using TD DFT methods are in agreement with the experimental results in great degree. According to Lambert-Beer theory, the molar absorption coefficient of MPTC (ϵ) was determined from the plot of absorbance vs concentration (Fig. S2 in Supplemental Materials). The ϵ value is obtained to be 2.338×10^5 L/mol/cm. In consideration of the particular structure of MPTC, it is speculated that MPTC should have larger fluorescence quantum efficiency due to it's fluorescent emission peak at 346 nm and is a prospective blue light emitting material in the organic light-emitting diodes[36].

3.4 Synthesis of RMPTC

A number of researches have shown that using the triazole motifs as the platform has potential application in the development of new fluorescence chemosensors. A variety of highly selective chemosensors for the detection of different metal ions (such as Fe^{3+} , Zn^{2+} , Cu^{2+} , Cd^{2+} and Hg^{2+} etc) based on click generated triazole have been designd and applied [37-40]. Especially, it is feasible and possible for MPTC to react with rhodamine B hydrazide in theory due to the triazole structure containing ester group [41]. By optimizing the experimental conditions, MPTC was designed and introduced to the rhodamine hydrazide to successfully synthesize a new rhodamine B derivative RMPTC through a serious of reactions (Scheme 1). The structure of RMPTC were characterized by ¹HNMR, ¹³C NMR, and

HRMS (Fig. S3, Fig. S4 and Fig. S5 in Supplemental Materials, respectively.). The results achieving the expected goal can provide reasonable guidance for synthetic reaction between similar 1,2,3-triazoles and rhodamine B.

3.5 Fluorescence spectra studies on response of RMPTC to metal ions

To determine the binding properties of RMPTC with metal ions, the fluorescence spectroscopy of RMPTC (50µM) in the presence of 15 kinds of metal ions such as Pb^{2+} , Mn^{2+} , K^+ , Na^+ , Ag^+ , Ca^{2+} , Cd^{2+} , Co^{2+} , Cu^{2+} , Fe^{3+} , Zn^{2+} , Ni^{2+} , Hg^{2+} , Li^+ and Mg^{2+} (40µM) was investigated respectively, and the results were displayed in Fig. 3A. It can be seen that RMPTC showed no obvious fluorescence emission with excitation at 563 nm in DMF/H₂O (v/v=1:1, Tris-HCl, pH=7.4) solutions, which is a typically characteristic peak of rhodamine B [15, 24]. However, upon binding to different metal ions, it was only observed that coordination of Hg²⁺ to sensor RMPTC resulted in a distinct fluorescence enhancement at 584 nm, while other metal ions showed no obvious fluorescence emission. Coupled with the fluorescent spectra changes, the emission color change from colorless to pink was clearly detected by naked eye (Fig. 3B inset), while other metal ions did not induce any apparent emission color change under the same conditions. In general, rhodamine spirolactam or spirolactone derivatives are nonfluorescent and colorless, whereas ring-opening of the corresponding spirolactam/lactone gives rise to strong fluorescence emission and a pink color. An appropriate ligand on a spirolactam ring can induce a color change as well as a fluorescence change upon addition of metal ions, even though this process is somewhat dependent on the solvent system[42]. Herein, it was attributed to ring opening from the spirolactam (RMPTC) to ring-opened amide (RMPTC-Hg²⁺ system) and RMPTC probably formed a novel compound with Hg²⁺. The result suggested that RMPTC can act as a sensor to detect Hg²⁺ through the distinct fluorescence "off-on" responses. Then the selectivity of RMPTC to Hg^{2+} over other metal ions were investigated by compared experiments (Figure 3(B)). It displayed that the addition of Hg²⁺ caused a 2900-fold increase in fluorescence intensity at 577 nm. The inset in Fig. 3(B) showed the photographs of the RMPTC in the presence of Hg^{2+} and other metal ions. It is obvious that other metal ions, such as Pb²⁺, Mn²⁺, K⁺, Na⁺, Ca²⁺, Cd²⁺, Co²⁺, Cu²⁺, Fe³⁺, Zn²⁺, Ni²⁺, Li⁺ and Mg²⁺ ions, did not induced any significant color and spectral change under identical conditions. The result demonstrated that the developed colorimetric sensor RMPTC possessed high selectivity for sensing Hg²⁺ due to the possible interaction between RMPTC and Hg²⁺.

Sensitivity is another very important criterion for a sensor. To investigate the sensitivity of RMPTC with respect to Hg^{2+} , fluorescence titration experiments were then performed as shown in Fig. 4. The fluorescence intensity of RMPTC (50µM) at 584 nm was gradually increased with the addition of increasing amount of Hg^{2+} ions, and consequently became saturated when the amount of Hg^{2+} reached 40µM. However, a continuous decrease in the fluorescence intensity was observed for further increments of Hg^{2+} concentration. The linear fluorescence enhancement of RMPTC upon addition of Hg^{2+} was obtained in the range of 2.00×10^{-5} - 4.00×10^{-5} mol/L (R² = 0.9442, Fig. S6 in Supplemental Materials). So it seems that RMPTC is not an ideal probe in quantitative determination of Hg^{2+} .

3.6 UV-vis spectra studies on response of RMPTC to metal ions

The UV-vis spectra of RMPTC (50 μ M) in DMF-H₂O (v/v=1:1, Tris–HCl, pH=7.40) were recorded in the presence of various metal ions as shown in Fig. 6A. It can been seen that there is the same trend as the fluorescence measurements. The free RMPTC also exhibited no absorption near 565nm, which is the characteristic absorbance of rhodamine B. However, upon addition of Hg²⁺ to the solution of RMPTC, a new absorption band appeared at 565nm along with the increasing intensity which was ascribed to the ring opened rhodamine moieties. Figure 6B shows the spectral variation of RMPTC upon the gradual addition of Hg²⁺ by the UV-vis titration experiments. A absorption band centered at 565 nm appeared and gradually increased with the gradual increase of Hg²⁺ ion concentration. When the concentration of Hg²⁺ was increased from 2.00×10⁻⁵ to 4.00×10⁻⁵ mol/L, the absorption peak intensity at 565nm gradually increased. But with the sequential addition of Hg²⁺, attainment of the plateau (the inset in Fig. 6B) implied saturation in the binding interaction between the two partners.

3.7 The competition experiments

To further identify a high selectivity of RMPTC as a fluorescent sensor, the competition experiments were performed in the presence of Hg^{2+} (50µM) mixed with other metal ion solutions with the same concentration (Fig. 7). It was noticed that the enhancement in fluorescence intensity resulting from the addition of Hg^{2+} was not affected too much by coexistence of other metal ions at this concentration. Meanwhile, only Hg^{2+} rendered significant fluorescence turn-on responses among all the tested metal cations, whereas all the coexistent metal ions had no noteworthy variance in the

fluorescence intensity. The results indicated that the fluorescence response of RMPTC towards Hg²⁺ was highly selectively than other competitive metal ions.

3.8 Effect of pH

The pH value is a crucial factor for the detection procedure. To evaluate the potential application of the sensor RMPTC in different environments, the effect of pH on the fluorescence intensity was investigated in a range of pH between 3.0-10.0. Fig. 8 is the effect of pH on the fluorescence intensity of RMPTC (plot a) and RMPTC-Hg²⁺ complex (plot b) with a concentration of RMPTC or Hg²⁺ fixed at 50µM. As shown plot a in Fig. 8, RMPTC showed obvious fluorescence when the value of pH is less than 4. But there was no any obvious and characteristic fluorescence observed for RMPTC in the range of pH from 4.0 to 10.0, which indicated the formation of stable spirocyclic RMPTC compound in the range of the value of pH. However, it can be seen from plot b in Fig. 8 that the fluorescence emission intensity of RMPTC was enhanced under the different value of pH from 4.0 to 8.0 in the presence of Hg^{2+} , which suggested that RMPTC- Hg^{2+} complex remain in a "turn-on" state within this pH range. And the maximum fluorescence response toward Hg^{2+} was observed at the value of pH as 7.4. If the value of pH is higher than 8.0, there was no fluorescence enhancement for RMPTC-Hg²⁺ system. The reasonable explanation is that the acidic medium(pH < 4.0) led to the dissociation of the complex and protonation of the ligand, and induced a decrease in emission intensity accordingly. However, at higher pH values (pH > 8.0), the OH⁻ ions and RMPTC compete for Hg²⁺ ion. And Hg²⁺ ions might bind to the OH⁻ and form the corresponding hydroxide precipitation Hg(OH)₂, which might decrease the actual concentration of Hg²⁺ ions in the sample solution and therefore cause the drop of fluorescence intensity^[43]. Therefore, a best response toward Hg²⁺ ions could be achieved at the pH 7.4. This result indicates that RMPTC can be used as a good fluorescent probe for Hg²⁺ ions determination in nearly neutral conditions typical of many biological system. Combined the results on the fluorescence and UV-vis titration measurements, RMPTC can be applied to highly selectively and qualitatively detect Hg²⁺ under physiological conditions, implying that we can further explore and make full use of RMPTC for detection of Hg²⁺ in BIOLOGICAL samples.

3.9 Time-response studies

A short response time is necessary for the fluorescent sensor to detect metal ions

in practical application. Therefore, the time dependence on the fluorescence intensity of RMPTC to Hg^{2+} was investigated by recording the change of the fluorescence intensity at 584nm after the addition of Hg^{2+} to RMPTC (50 μ M) in DMF-H₂O (v/v=1/1, pH 7.4) buffer solution (Fig.9). As shown Fig. 9, the fluorescence intensity of RMPTC-Hg²⁺ system at 584nm gradually increased and reached the maximum over a period of 3 min and remained unchanged within 12h, which indicated that the reaction between RMPTC and Hg^{2+} was completed within 3 min. So the right response time is 3 min which can be used to do the relevant spectroscopic experiments. It demonstrated that sensor RMPTC could be used for the real-time analysis of Hg^{2+} in aqueous media.

3.10 Determination the binding stoichiometry of RMPTC and Hg^{2+} complex

It is reported that rhodamine B and triazoles based derivatives can form the complexs with different metal ions by displaying special color and various complex ratio [37, 39]. To investigate the binding stoichiometry between RMPTC and Hg²⁺ ion, the method of equimolar continuous variations (Job's plot) [44] were therefore carried out by maintaining the total concentration of RMPTC and Hg²⁺ constant (50 μ M) and changing the mole fraction of RMPTC from 0 to 1. The result indicated that the fluorescence intensity at 584nm of the RMPTC-Hg²⁺ complex was achieved at a mole fraction of approximately 66.7% of Hg²⁺ ions, indicating that a 1:2 stoichiometry was the likely binding mode of complex RMPTC with Hg²⁺ ions (Fig. 5).

In order to confirm whether our experiment is correct or not, we decided to calculate molecular orbitals of RMPTC-Hg²⁺ complex. Based on Gaussian 09 program package, a plausible structure of RMPTC-Hg²⁺ complex was generated from the available calculated data to demonstrate the reasonableness of 1:2 stoichiometry for RMPTC binding to Hg²⁺ ions. And the molecular structure of RMPTC-Hg²⁺ complex was fully optimized and its structural energy was calculated by using B3LYP method in terms of LANL2TZ basis set for Hg and 6-311G(d) basis set for H, C, N, O and F respectively. The corresponding results were shown in Fig. 5B and Table S3. In Fig. 5B, RMPTC behaved as two pairs of tongs, one is the ligand using one oxygen atoms from carbonyl group and one nitrogen atom from triazoles group to bind one Hg²⁺ ion, another one is the ligand using one oxygen atoms and one nitrogen atom from hydrazide group to bind another Hg²⁺ ion. The molecular docking diagram showed that RMPTC can provide suitable space to accommodate two Hg²⁺ ions with two penta cyclic groups formed. The calculated data in Table 1 indicated that the

formation of RMPTC-Hg²⁺complex as 1:2 stoichiometry caused the energy difference of $\Delta E(a.u.)$ and $\Delta E(ev)$, which were more small than that of as 1:1 stoichiometry. The results illustrated that RMPTC binding with two Hg²⁺ ions was more stable than that of one Hg²⁺ ion and confirmed theoretically the experimental result.

3.11 Proposed response mechanism of RMPTC to Hg^{2+}

When designing florescence sensor systems (the host) for the recognition of small molecules such as cations, anions, or amino acids (the guest), it is significant to utilize specific chemical interactions or host-guest reactions that cause a change in the luminescent emission properties of the system. If the specific interaction between the host and the guest is noncovalent and reversible, and the interaction can be disrupted under certain conditions, the indicator is referred to as a chemosensor[45-47]. A large number of studies related to fluorescent chemosensors based on spiroring-opening processes of rhodamine have been published [24,37,38,42]. If the binding interaction between the host and the guest is based on an irreversible chemical reaction, the indicator is described as a chemodosimeter. For chemodosimeters, it is necessary that there are at least two functional units. One of two units is the reaction site, in which the host binds to the detection object. And the second unit is responsible for a spectroscopic signal that relies on the binding to the analyte. In principle, both the interaction between the analyte and the chemodosimeter and the variation in detectable signal are irreversible[45]. Generally, rhodamine derivative shows a red color change and strong fluorescence in acidic solutions by activation of a carbonyl group in a spirolactone or spirolactam moiety. In a similar way, an appropriate ligand on a spirolactam ring can induce a color change as well as a fluorescence change upon addition of metal ions, even though this process is somewhat dependent on the solvent system [42].

In the present study, based on the above spectral analysis and molecular docking, a plausible binding mode of RMPTC•Hg²⁺complex is depicted in Scheme 2. The rhodamine derivative RMPTC is nonfluorescent and colorless, which exhibited the typical closed ring effect for rhodamine B units due to the combination of MPTC. The interaction of RMPTC with Hg^{2+} made rhodamine B ring opened with two penta cyclic groups formed. The reaction site of chemodosimeter RMPTC, as the first unit, was located in oxygen atom of carbonyl group and nitrogen atom of triazole group of MPTC unit, which bound to one Hg^{2+} ion. The second unit providing the spectroscopic signal was situated in nitrogen atoms and oxygen atom of rhodamine B

hydrazide unit, which was responsible for interacting with another Hg^{2+} ion. This binding mode has been confirmed by the fluorescence measurement observed and structural computation as the induced conformational alterations of RMPTC upon 1:2 complexation with Hg^{2+} . The complex of RMPTC with Hg^{2+} was verified by ESI–MS testing (Fig.10). It can be seen that there is a strongest peak at m/z 996.6 , which probably is assigned to [Hg+Na+2Cl+2CH₃OH-4H] with less feasibility. However, a peak at m/z 1081.6 has stronger intensity, which seems more reasonable and can be assigned to [RMPTC+2Hg+ K-H]. The observed and calculated isotopic patterns agree well with each other(Fig.10, inset). Combined the results on the spectral experiment and computed calculation, the present ESI-MS detection confirmed the formation of RMPTC-Hg²⁺ complex in 1:2 ratio again. Thus, the interaction of RMPTC with Hg^{2+} could trigger the formation of the ring-opened form of RMPTC from the spirolactam form. Moreover, RMPTC can serve as a "naked-eye" chemodosimeter targeted toward Hg^{2+} , similar to some reports on rhodamine-based fluorescence probes for specific recognition of metal ions [48-50].

4. Conclusion

In summary, one new of the 1,2,3-triazoles, 5-methyl-2-phenyl-2H-1,2,3 -triazole-4-carboxylic acid (MPTC), was investigated in terms of structural, fluorescence spectroscopic, UV-visible spectroscopic and theoretical analysis by using HF/6-31G(d), CIS/6-31G(d) and B3LYP/6-31G(d) methods. The experimental results are in line with the theoretical values derived from structural parameters, providing guidance for utilizing MPTC in photophysical chemistry due to its special optical property. Furthermore, a novel rhodamine-based colorimetric and turn-on fluorescent chemodosimeter RMPTC was successfully developed and its sensing ability for a wide range of metal ions was investigated. It selectively responds to Hg^{2+} by enhancement of the fluorescence and UV-vis absorbance intensity with 1:2 stoichiometry and also facilitates "naked-eye" detection of Hg^{2+} . The results demonstrate that RMPTC has a promising future for reliable and qualitative detection of Hg^{2+} ions in aqueous solutions under physiological conditions. It is also anticipated that the chemodosimeter RMPTC will significantly promote the studies on the effects of Hg^{2+} ions in biological systems.

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Figure captions:

Scheme 1 Synthesis route of RMPTC

- **Scheme 2** Proposed recognition mechanism of RMPTC towards Hg^{2+} .
- Fig.1 (A) The optimized structure of MPTC; (B1) Frontier Molecular Orbital of ground state on LUMO; (B2) Frontier Molecular Orbital of ground state on HOMO;
- Fig. 2 (A) The electronic absorption spectra of MPTC predicted by TD-DFT calculations. Inset: the absorption spectra of MPTC measured in methanol.
 (B) The fluorescence emission spectra of MPTC predicted by TD-DFT calculations. Inset: the fluorescence emission spectra of MPTC measured in methanol.
- **Fig. 3.** Fluorescence spectra (A) and fluorescence intensities (B) at 584nm of RMPTC (10μ M) in DMF-water (v/v=1/1, Tris-HCl, pH 7.4) in the presence of various cations (40μ M) (λ_{ex} =563 nm). Inset: Photographs of the RMPTC in the presence of Hg²⁺ and other metal ions.
- **Fig. 4** Fluorescence spectra ($\lambda ex=563 \text{ nm}$) of RMPTC (10 μ M) upon the addition of Hg²⁺ in DMF-water (v/v=1/1, Tris-HCl, pH 7.4). The illustration: graph of the fluorescence intensity at 584 nm as a function of Hg²⁺ concentration.
- **Fig. 5** (A)The Job plot of RMPTC with Hg^{2+} ion using fluorescence.
 - (B) The optimized structure of RMPTC binding to two of Hg^{2+} ions.
- **Fig. 6A** Absorption spectra of RMPTC (50μM) in the presence of different metal ions (40μM) in DMF-water (v/v=1/1, Tris-HCl, pH 7.4)
- Fig. 6B Absorption spectra of RMPTC (50 μ M) upon the addition of Hg²⁺ (0-50 μ M) in DMF-water (v/v=1/1, Tris-HCl, pH 7.4).
- Fig. 7 Fluorescent selectivity of RMPTC (50 μ M) toward Hg²⁺ over various competitive metal ions (5.0 μ M) in DMF-water (v/v = 1/1, Tris-HCl, pH7.40). The black bars represent the intensity of the solution upon the addition of Hg²⁺(50 μ M).The red bars represent the intensity of RMPTC in the presence of other metal ions(50 μ M) and Hg²⁺(50 μ M).
- **Fig. 8** Effect of pH on the fluorescence intensity of RMPTC (a) and RMPTC-Hg²⁺ complex (b).
- Fig. 9 Time response of RMPTC to Hg^{2+} in DMF-water (v/v = 1/1, Tris-HCl, pH7.40) from 0 to 60min.
- Fig. 10 (A) ESI-MS of RRMPTC in the presence of Hg^{2+} and trace amounts of Cl⁻.

Inset: enlarged patterns for the [RMPTC+2Hg+ K-H].

(B) The calculated ESI-MS of RMPTC in the presence of Hg²⁺ and Cl⁻. Inset: calculated isotopic patterns for the [RMPTC+2Hg+ K-H].

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	Energy change				
RMPTC:Hg ²⁺	E _{REMPC} (a.u.)	$E_{\rm Hg}^{2+}({\rm a.u.})$	E _{RMPTC:Hg2+} (a.u.)	$\Delta E(a.u.)$	$\Delta E(\mathrm{ev})$
1:1	-2080.91	-41.79	-2123.15	-0.44	-12.11
1:2	-2080.91	-83.59	-2165.02	-0.53	-14.37
		R			

Table 1	Comparison on energy changes of RMPTC binding to Hg ²⁺ using the
	B3LYP functional in Gaussian 09.



Scheme 1 Synthesis route of RMPTC



Scheme 2 Proposed recognition mechanism of RMPTC towards Hg²⁺.

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Fig.1 (A) The optimized structure of MPTC; (B1) Frontier Molecular Orbital of ground state on LUMO; (B2) Frontier Molecular Orbital of ground state on HOMO.



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Fig. 3. Fluorescence spectra (A) and fluorescence intensities (B) at 584nm of RMPTC (10 μ M) in DMF-water (v/v=1/1, Tris-HCl, pH 7.4) in the presence of various cations (40 μ M) (λ_{ex} =563 nm). Inset: Photographs of the RMPTC in the presence of Hg²⁺ and other metal ions.



Fig. 4 Fluorescence spectra ($\lambda ex=563 \text{ nm}$) of RMPTC (10 μ M) upon the addition of Hg²⁺ in DMF-water (v/v=1/1, Tris-HCl, pH 7.4). The illustration: graph of the fluorescence intensity at 584 nm as a function of Hg²⁺ concentration.



Fig. 5 (A) The Job plot of RMPTC with Hg^{2+} ion using fluorescence. (B) The optimized structure of RMPTC binding to two of Hg^{2+} ions.



Fig. 6A Absorption spectra of RMPTC (50 μ M) in the presence of different metal ions (40 μ M) in DMF-water (v/v=1/1, Tris-HCl, pH 7.4)



Fig. 6B Absorption spectra of RMPTC (50 μ M) upon the addition of Hg²⁺ (0–50 μ M)

in DMF-water (v/v=1/1, Tris-HCl, pH 7.4).



Fig. 7. Fluorescent selectivity of RMPTC (50 μ M) toward Hg²⁺ over various competitive metal ions (5.0 μ M) in DMF-water (v/v = 1/1, Tris-HCl, pH7.40). The black bars represent the intensity of the solution upon the addition of Hg²⁺(50 μ M).The red bars represent the intensity of RMPTC in the presence of other metal ions(50 μ M) and Hg²⁺(50 μ M).



Fig. 8. Effect of pH on the fluorescence intensity of RMPTC (a) and RMPTC-Hg²⁺ complex (b).



Fig. 9. Time response of RMPTC to Hg^{2+} in DMF-water (v/v = 1/1, Tris-HCl, pH7.40) from 0 to 60min





(B) The calculated ESI-MS of RMPTC in the presence of Hg^{2+} and Cl^{-} . Inset: calculated isotopic patterns for the [RMPTC+2Hg+ K-H].





Graphical abstract

Highlights

- Analyzing the structural and spectral properties of MPTC.
- MPTC was synthesised a novel rhodamine based fluorescent chemosensors RMPTC.
- RMPTC selectively responded to Hg²⁺ as 1: 2 molar ratios by "off-on" mechanisms.

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